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The Use of the Hemagglutination-Inhibition Reaction for the Diagnosis of Small Pox (Variola Vera).

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Serum investigations occupy a definite place among the laboratory-diagnostic methods of smallpox.

The most widely used of these at present is the complement fixation test. This test can be used for an early diagnosis (determination of antigen) as well as for a later, retrospective one (antibody development). It should be emphasized however, that the complement fixation test, while giving a high percentage of advantageous answers, will not allow a differential diagnosis between small pox and vaccine (Ref 1).

In 1950 Collier and co-authors (Ref 2) proposed a new method of serological diagnosis for smallpox- by the antihemagglutinin level in the serum of smallpox patients, as determined in the hemagglutination-inhibition reaction. The numerous observations of these authors, conducted during a latge outbreak of smallpox on the island of Java, showed that the people sick with smallpox have high antihemagglutination titers considerably exceeding those of the recently vaccinated. This difference allows us to differentiate smallpox from vaccine in an overwhelming majority of cases.

We have investigated the hereto unapplied laboratory-diagnostic methods of this disease. In addition to the isolation of the smallpox virus in chicken embryos, we used the antibody titer (antihemagglutinins) in sick people's blood serum as a diagnostic method.

To procure the serum,2-5 ml of blood was taken, with asceptic precautions from the patients ulnar vein. In individual cases, when it was impossible to take blood from a vein it was taken from a finger in the amount of 0.2 ml and transferred to a test tube containing 1.8 ml of a sterile physiological solution. The mixture was centrifuged to free it from formed elements. The serum dilution received in these cases were congruently 1 : 20. The sera were protected in sealed ampules under refrigeration until the analysis. The investigation of the sera in the hemagglutination-inhibition reaction was conducted single-purposedly. Before the arrangement of the reaction, the sera were heated in a hot water bath at 58° for 20 minutes. The hemagglutination-inhibition reaction was established in a 1 ml amount with 4 agglutinating units of the vaccines virus (cultivated on the chorion-allantoic membrane of a chicken embryo) and a 1% suspension of chicken erythrocytes. Before the addition of the erythrocytes, the mixture of the virus with the serum was sustained 40 minutes at room temperature. The reaction was read within

30 minutes after the addition of the erythrocytes. The end dilution which caused complete suppression of the erythrocytic agglutination by the virus was adopted for the titer of the serum.

In all, 46 sera were investigated. Included in these are sera that were retaken from some of the patients at different periods after the start of the disease (Table 1).

Table 1 shows the definite relation between the time progression of the disease and the level of antihemagglutinins in the serum. The highest antihemagglutination titers in the sick persons are recorded during the period between the 12th and 26th day of the disease, with a particularly sharp rise between the 12th and 15th day. After this, the level of antihemagglutinins in the smallpox patient's serum gradually decreases.

In conformity with the measure of the antihemagglutination titer, all the patients examined by us can be conditionally divided into three basic groups, depending upon the different time progressions from the start of the disease. The first-from the 6th to the 10th day; the second--from the 12th to the 26th day; and the third-from the 27th to the 60th day. As from the 6th day through the 10th day of the disease, the antihemagglutination titers varied from 40 to 640, from the 1st through the 26th day-from 20 to 5,120 and from the 27th through the 60th day-from 20 to 320. In the first group the titer 640 was detected in only one of the six patients, in the others the maximum titer never exceeded 160. In the second group low titers were detected in only three of the sixteen patients (in one-20, in two-160). In the third group(from the 27th through the 60th day of the disease) only one of the twenty-four patients' titers reached 320, seventeen (the majority) reached 160 and the rest were lower.

In addition to the data received, a small group of recently vaccinated, healthy people who were in close and prolonged contact with the patients were examined by us as a control. The examinations just mentioned were made 2-4 weeks post vaccination, i.e., in the period corresponding to the maximum accumulation of antihemagglutination titer in vaccinated persons (Downie, 1951). As seen from the observational data of this group (Table 2), the antibody level in 6 of the 7 persons was from 0 to 80 and amounted to 160 in only one.

The data presented above, concerning the accumulation rate of antihemagglutinins in freshly vaccinated people who are in contact with smallpox patients, agree completely with the results of our earlier serum investigations of children after their initial vaccinations. The data also show that the quantity of antihemagglutinins in the smallpox patients markedly exceed that in recently vaccinated persons.

On the basis of an analysis of the material received, it is proper to acknowledge that the determination of the antihemagglutination titer may be a valuable laboratory-diagnostic discernment method for smallpox, particularly in unvaccinated persons or those vaccinated many years previously. In unvaccinated persons, or in those subjected to vaccination many years previously, antihemagglutinins in the serum are either absent

or their titer is very low (Ref 1, 3). Considering this, the presence of even comparatively low titers (80-160) in these cases can help to establish a diagnosis of smallpox.

As indicated above, the maximum antihemagglutination titer recorded in the recently vaccinated persons did not exceed 160. Evidently, it may be even a little higher in isolated cases, as we once discovered a titer of 320 among the sera of initially vaccinated children. In connection with this, in those cases where the sick person has been subjected to vaccination or revaccination shortly before the start of the disease, it is necessary to consider the antihemagglutination level and also the progression of time since the start of the disease and since the inoculation. In such cases, only high antihemagglutination titers (640 and higher) can serve as a basis for making a smallpox diagnosis.

As shown by Table 1, the antihemagglutination level at the start of the disease may not be sufficiently high to allow a diagnosis. In these cases it is expedient to resort to re-investigation of the serum by the hemagglutination-inhibition reaction. An appreciable increase of the antihemagglutination titers in the second serum will permit confirmation of a smallpox diagnosis. For an example, the results from a re-examination of two patients, at different stages of the disease, are shown in Table 3. These results clearly show the dynamics of the antihemagglutination rise in the course of the disease.

It is necessary to note however, that in several smallpox patients the antihemagglutination titers remain low even in the period characterized by their maximum content (See Table 1). Therefore, the presence of low titers does not allow the exclusion of a smallpox diagnosis.

The results of the completed tests attest to the fact that the method of investigation of smallpox patients' serum in the hemagglutination-inhibition reaction indisputably deserves serious attention, and together with the clinical, epidemiological and other laboratory tests must find a use with the diagnostics of this disease. The large advantages of the indicated method are its simplicity, availability and its quickness in producing an answer.

References

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2. Collier, W. A., Schoenfeld, J. K.- Med Jour Australia, 1950, v. 2, p. 363-366.

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Table 1.

Antihemagglutination Titers in Smallpox Patients in Relation to the Day of the Disease.

Serum No.	Name of Patient	Age	Prior Vaccination	Course of Disease	Time in Days from start of disease	AH Titer
1	All.	16	V. in childhood	Medium	6	80
2	Nam.	28	V. & revaccinated	Varioloid	8	640
3	Ut-v	15	Not vaccinated	Heavy	8	40
4	Nar.	9	" "	"	9	160
5	N-va	4	" "	"	9	40
6	N-va	5	V. in childhood	Varioloid	10	160
7	Ai-va	15	V. & revaccinated	Heavy	12	1280
8	D-v	5	Not vaccinated	Medium	13	640*
9	Kh-va	8 mos	" "	Heavy	14	5120
10	All.	16	V. in childhood	Medium	14	640
11	D-v	3	Not vaccinated	"	15	2560
12	Er-va	38	" "	Heavy	15	640
13	Khud-v	28	V. in childhood	Medium	15	640
14	Al.	18	" " "	Medium	17	160
15	Khad.	23	" " "	Varioloid	18	20
16	Dzh.	3	Not vaccinated	Medium	20	320
17	B-v	5	" "	"	24	160
18	Rakh-va	26	V. & revaccinated	Heavy	24	320
19	B-v	15** days	Not vaccinated	Medium	25	640
20	D-v	5	" "	"	26	640
21	Nar.	9	" "	Heavy	26	640
22	Nar-va	5 mos	" "	Medium	26	640
23	U-va	8	V. in childhood	"	27	160
24	N-va	5	" " "	Varioloid	27	160
25	Kol-v	16	V. in childhood	Heavy	27	160
26	Kar-v	27	" " "	Medium	29	160
27	Mur-va	23	" " "	"	29	160
28	Rakh-v	27	" " "	Varioloid	29	80
29	Dzh-va	5	Not vaccinated	Medium	29	160
30	Mur-v	19	V. in childhood	"	30	160

16	Dzh.	3	Not vaccinated	Medium	20	320
17	B-v	5	" "	"	24	160
18	Rakh-va	26	V. & revaccinated	Heavy	24	320
19	B-v	15** days	Not vaccinated	Medium	25	640
20	D-v	5	" "	"	26	640
21	Nar.	9	" "	Heavy	26	640
22	Nar-va	5 mos	" "	Medium	26	640
23	U-va	8	V. in childhood	"	27	160
24	N-va	5	" " "	Varioloid	27	160
25	Kol-v	16	V. in childhood	Heavy	27	160
26	Kar-v	27	" " "	Medium	29	160
27	Mur-va	23	" " "	"	29	160
28	Rakh-v	27	" " "	Varioloid	29	80
29	Dzh-va	5	Not vaccinated	Medium	29	160
30	Mur-v	19	V. in childhood	"	30	160
31	Khal-v	24	V. & revaccinated	"	30	160
32	Um-v	22	V. in childhood	"	30	160
33	Um-va	58	V. w/negative results	"	32	160
34	Nar-va	5	Not vaccinated	"	32	160
35	B-v	5	" "	"	33	40
36	Bur.	3	" "	Heavy	33	320
37	Rakh-va	26	V. and revaccinated	"	33	160
38	B-va	5	Not vaccinated	"	36	160
39	Kur-va	21	V. in childhood	Medium	38	160
40	Shab-v	25	" " "	"	39	160
41	Tur-va	26	" " "	Varioloid	40	160
42	Bur.	3	Not vaccinated	Heavy	43	160
43	B-v	2	" "	Medium	48	80
44	Sad-va	18	V. in childhood	"	48	20
45	Um-va	26	V. & revaccinated	Heavy	54	40
46	Dul-v	24	Not vaccinated	"	60	40

*Dilutions were not investigated past this point.

**Age shown is that at the onset of the disease.

Table 2.

Antihemagglutination Titers in Healthy, Freshly Vaccinated Persons Who Have Had Contact With Smallpox Patients.

Name	Age (in years)	AH Titers
Nar-va	28	40
Om-Baim	37	40
Bur-va	23	160
Khur-va	28	80
B-v	13	40
Khal-va	33	0
Um-va	35	10

Table 3.

The Rise of Antihemagglutinins in the Process of Smallpox.

Serum No.	Name	Day of Disease	AH Titer
1	Nar.	9th	160
2	" .	26th	640
3	All. D	6th	80
4	"	14th	640